*Journal of Chromatography*, 245 (1982) 144–147
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,969

## Note

# Separation of thiamin and its common degradation and oxidation products by high-performance liquid chromatography

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(Received March 23rd, 1982)

Thiamin in foods, feeds and pharmaceutical preparations becomes oxidized and degraded upon aging and contact with other chemicals. It is important to determine the degradation products of thiamin in urine and body fluids to clarify the thiamin metabolism. However, a reliable method to determine the concentration of these compounds has not been exploited. In recent years, high-performance liquid chromatographic (HPLC) techniques have been introduced for thiamin and its phosphate esters[1–5].

In this paper, we describe an HPLC method capable of determining thiamin, thiamin disulphide (TDS), 2-methyl-4-amino-5-aminomethylpyrimidine (AMPy), 2-methyl-4-amino-5-hydroxymethylpyrimidine (HMPy), 4-methyl-5-(β-hydroxyethyl)-thiazole (Tz) and thiochrome (Thc). These thiamin derivatives are commonly found as products of various degradation and oxidation reactions of thiamin[6–12].

## EXPERIMENTAL

### *Reagents*

Thiamin hydrochloride was obtained from Wako (Osaka, Japan); AMPy dihydrochloride, HMPy, TDS, Thc, Tz and thiamin monophosphate (TMP) were donated by the Central Research Division of Takeda Chemical (Osaka, Japan). All other chemicals were of the best grade commercially available.

### *Apparatus*

The following were used: LC-3A pump for liquid chromatograph; SIL-1A injector; Shodex OH pak M614 column (25 × 0.6 mm I.D.); CTO-2A column oven (35°C); SPD-2A UV detector; flow cell (10 × 1 mm I.D.); Chromatopac C-RIA recorder (chart speed 2 mm/min). All of the equipment was purchased from Shimadzu (Kyoto, Japan).

### *Procedure*

For HPLC, the mobile phase (0.05 *M* sodium phosphate-phosphoric acid

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0021-9673/82 0000–0000/$02.75 

buffer pH 3.75) was pumped at a flow-rate of 0.5 ml/min. A 10-$\mu$l volume of sample was loaded on the sample loop and then injected on to the column, the zero time being noted. The absorbance at 250 nm of the column effluent was monitored with a spectrophotometer and recorded graphically.

## RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram obtained for a standard solution containing $5 \times 10^{-4}$ *M* of each of TMP, AMPy, HMPy, Thc, Tz, thiamin and TDS by use of the above procedure. The easy and clear separation of TMP from thiamin by HPLC has not been reported so far. For the separation of the overlapping peaks, HMPy (3) and Tz (5) can be moved to the left or right by decreasing or increasing the pH in the mobile phase by 0.2 units as indicated by the arrows in Fig. 1. Also, thiamin and TDS can be independently and fluorometrically quantitated by post-column conversion into fluorophores by the method described previously[3]. The separation of TMP, AMPy, HMPy and thiamin on a 25-cm $\mu$Bondapak $C_{18}$ column under the same conditions was excellent.

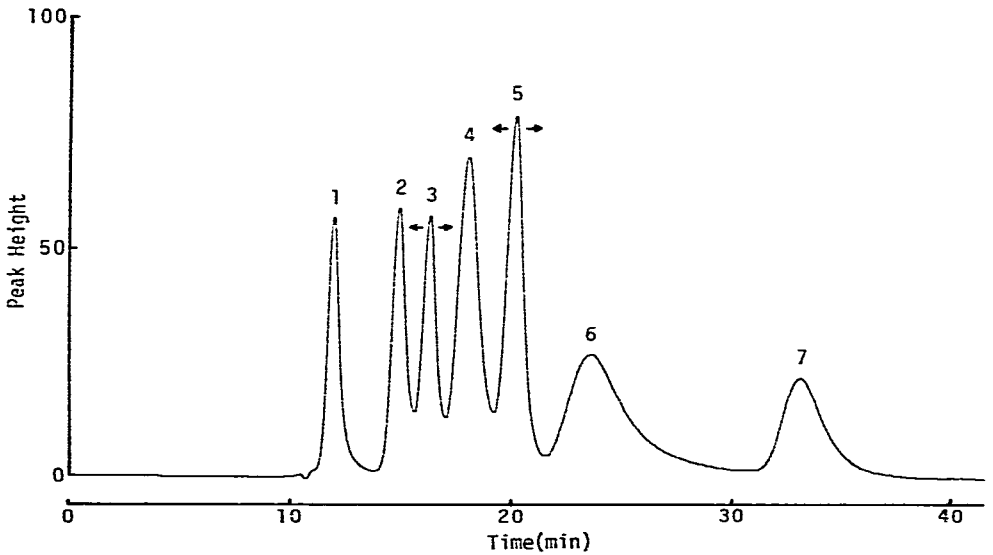


Fig. 1. Elution profiles of thiamin and its degradation and oxidation products. Peaks: 1 = TMP; 2 = AMPy; 3 = HMPy; 4 = thiamin; 5 = Tz; 6 = TDS; 7 = Thc. Arrows indicate movement of peaks 3 and 5 to the left or right relative to peak 4 on decreasing or increasing the pH respectively.

Monitoring of the absorbance at 250 nm was performed because AMPy, HMPy and Tz have maximum absorption in this region and the absorbance of thiamin, TDS and Thc at this wavelength is comparable to that at 275 nm.

This procedure achieved the quantitation of these compounds down to 10 ng for TMP, AMPy, HMPy, thiamin and Tz, and 30 ng for TDS and Thc. A linear relationship between amount and peak height was obtained for each compound up

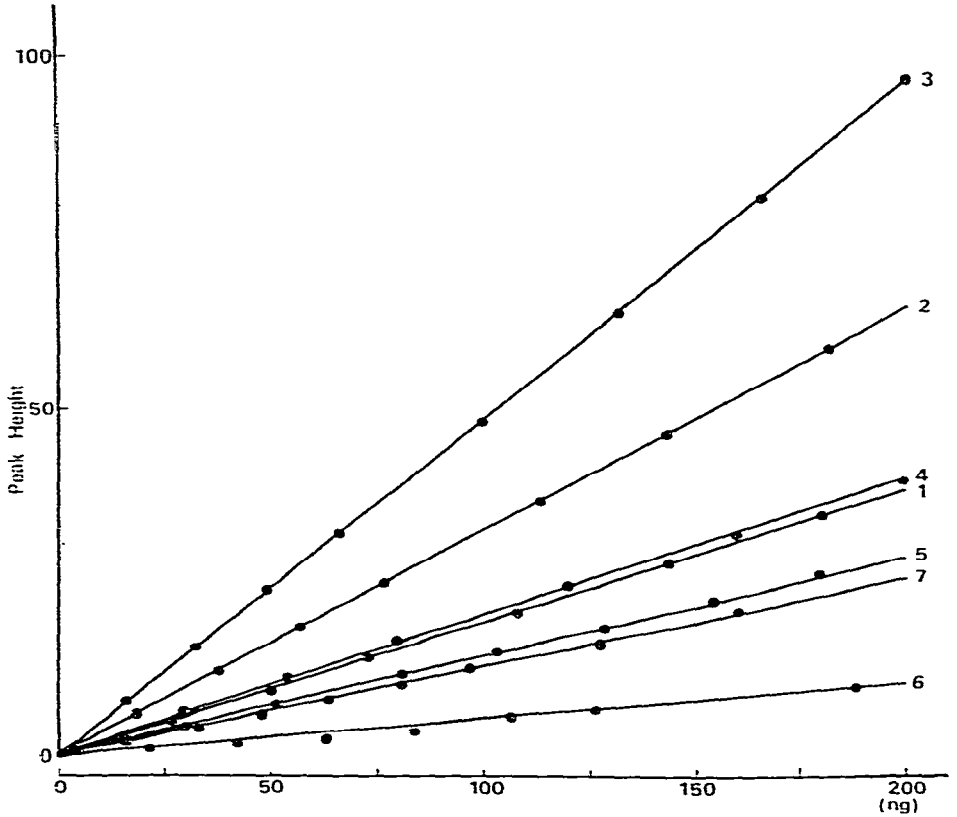


Fig. 2. Calibration graph obtained for thiamin and its degradation and oxidation products.

to 200 ng. (Fig. 2). This method has great advantages over more conventional methods both in time saved and accuracy achieved. We are currently quantifying and identifying products arising from thiamin after exposure to various chemicals and physical conditions.

ACKNOWLEDGEMENTS

We are grateful to Dr. Y. Oka, Takeda Chemical Co. for provision of chemicals. This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan and the scientific cooperation between the Japanese Society for the Promotion of Sciences and the National Research Council of Thailand. The authors extend their gratitude to Dr. J. P. Matthews, Kyoto University, for assistance in preparation of the manuscript.

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